Titre de l'étude: A prospective pilot study to evaluate the feasibility of prolonged fasting for the treatment of Long COVID patients

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1. INTRODUCTION

1.1. Rationale

There are more than 400 M of individuals worldwide have not fully recover to their previous level of health following an infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] These individuals developed persistent symptoms that are now referred to as Long COVID (LC). LC is a complex and multisystemic condition potentially affecting any organ in the body. LC is characterized by persistent disabling fatigue, nonrestorative sleep, post-exertional malaise, and neurocognitive impairment, although many other symptoms have been reported.[2] Diagnosis is clinical and based on symptom reports owing to the absence of diagnostic biomarkers. LC has emerged as a major public concern in this post-pandemic era. A recent survey by Statistics Canada showed that 3.5 M Canadians have LC and almost 50% still have symptoms after a year, and most are unable to work.[3] Another study from Quebec showed that health care workers that reported at least one COVID-19 episode have an overall risk of 12% of developing LC.[4] LC-associated symptoms can have a debilitating functional impact, significantly impairing health-related quality of life leading to anxiety and depression.[5] LC is associated with a huge societal burden as symptoms may last for years. Most importantly, there is no validated treatment for this condition.

The complexicity of the pathogenicity have hampered the rapid development of efficient pharmalogical treatments. As an alternative, non-pharmalogical approaches like fasting, which acts on multiple metabolic pathways, are being explored. Prolonged fasting (PF) is defined as voluntary renouncement of food intake for 7 days. Although robust data are lacking, based on case reports, small case series and personal clinical experience, PF have been associated in some LC patients with significant symptoms improvement, ability to return to work and even apparent clinical cure.[6,7]

There is an urgent need for treatment in LC patients. Many have been suffering for years now with little clinical improvement. PF may represent a novel, cheap and efficient approach to improve the health-related quality of life (HRQoL) for these patients.

1.2. Literature review

The SARS-CoV-2 virus primarily enters the cells through interaction between the viral spike protein and the angiotensin-converting enzyme-2 (ACE2) receptor, which is expressed by various cell types in the body, leading to an inflammatory response that impairs organ function.[8] The underlying mechanisms leading to LC is not fully understood but may involve chronic inflammation, oxidative stress, microclotting, production of auto-antibodies, dysfunction of complement response, brian blood barrier permeability or viral persistence.[8] Oxidative stress can lead to mitochondrial dysfunction.[9]

There is no consensus on therapeutic approaches to address LC. Fasting has been used by various cultures as a therapeutic modality. Prolonged fasting (PF) is defined as voluntary abstention of food intake lasting > 5 days. PF has shown multiple effects on pathological mechanisms involved in LC, such as decreasing inflammation or reducing oxidative stress.[10,11] Furthermore, PF has been shown to reduce risk factors associated with severe COVID-19 courses.[12] This includes weight loss, reduction in waist circumference,[13] normalization of blood pressure,[16] as well as glucose and lipid levels.[14,15] which are linked to COVID-19 comorbidities like obesity, hypertension, diabetes or dyslipidemia.

Data on the effect of fasting in LC are lacking. The is currently no undergoing clinical trial on fasting in LC. Further rational for evaluating the effect of fasting comes from clinical observation of patients which self decided to perfom PF and reported improvement of their condtion, and from case report or small case series.[6,7].

2. HYPOTHESIS, GOAL AND OBJECTIVES

We hypothesize that in home supervised PF is feasible and may improve symptoms and health-related quality of life (HRQoL) in patients with LC.

Primary objective:

- To assess the fesability and acceptability of in home supervised PF (7 days) in 25 patients with severe LC

The secondary objectives are:

- To assess mean changes from baseline to one month post-PF on the SF 36 physical component score questionnaire (PCS)

- To assess mean changes from baseline to one month post-PF in functional status as measured by the Post-COVID functional status scale

  To assess mean changes from baseline to one month post-PF in LC-associated fatigues as measured by the Fatigue Severity Score questionnaire

- To assess mean changes from baseline to one month post-PF in neurocognitive status as measured by the Cognitive failure questionnaire version 2.0

3. METHODOLOGY

3.1 Study design

To address our primary goal, we propose a prospective, open label, single-center, interventional pilot study in patients with LC. Participants with severe LC, as defined here by a post-COVID functional status scale score (PCFS) of > 2, will undergo in home PF for 7 days under medical surveillance. Patients with severe LC are prioritized because they are the ones that will most likely benefit from a new intervention as there are the less likely to improve overtime. Indeed, data have shown a < 10% improvement after a year post-infection in these patients.[17] Patient-reported outcome measures (PROMs) will be assessed at baseline, at day 7, and at one month post-PF.

3.2 Sample size

It is hoped that the data collected by this study will contribute to the design for a larger clinical trial. Based on Lewis et al.'s recommendations for sample size calculation in feasibility studies,[23] we defined two calculations: one for retention and one for acceptability. For participantetention, an unacceptable threshold was set at $\leq$ 50%, with an expected value of 80%. For acceptability, an unacceptable threshold was set at 70%, with an expected value of 90%, resulting in sample sizes of 18 and 25, respectively. Results were obtained using G*Power 3.1.9.4. Based on the number of LC patients in active care at our clinic (> 700), recruiting 18-25 participants with severe LC at our clinic is feasible.

3.3 Study population

This study focuses on patients with severe LC who have no exclusion criteria for PF. All participants (> 18 year-old) will be recruited at the CIUSSSE-CHUS post-COVID clinic. Our clinic sees 10-15 new patients per week and we have over 700 patients in active care. Most of these patients have severe LC. Recruting up to 25 patients for this study within 6 months poses no problem.

3.4 Eligibility and screening

Because of reference bias (to be seen at the clinic, patients must be referred by a health care professional), most (> 80%) patients seen at the clinic have severe LC based on PCFS scores of 3 or 4. All new patients seen at our clinic are evaluated for their functional status with the PCFS tool as part of standard care. Potential participants will be screened by the principal investigator prior to entry into this study. If interested, an explanation of the study and discussion of the expected side effects, and full disclosure of the “informed consent” documents will take place by the study nurse. Participants will be recruited sequentially. Participants will continue to take their regular medication during PF.

Inclusion criteria:

- Age 18-64 years
- Diagnosis Long Covid based on WHO criteria (post-acute COVID-19 symptoms persisting ≥12 weeks) and validiated by an experienced cinician

- Body Mass Index between 18.5 to 39 kg/m2)
- Able to communicate in and comprehend English and/or French language
- Present written / signed declaration of consent
- Ability to understand the patient information and willingness to sign the consent form
- Willing to limit physical activity during PF

Exclusion criteria:

- Current underweight condition (body mass index less than 18.5 kg/m2) or weight loss exceeding 3 kg within the last month or 5 kg within the last three months.
- Current or history of eating disorder (e.g., anorexia, bulimia).
- Psychiatric condition that limits understanding of the examination protocol (unable to consent)
- Participation in another intervention study.
- Fasting during the last six months
- Pregnancy or breastfeeding status.
- Diagnosis of chronic inflammatory bowel diseases, celiac disease or colorectal cancer according to the guidelines of the Canadian Society of Gastroenterology
- Use of anti-psychotic drugs
- Start of novel drug therapy for long COVID
- Contraindication for additional blood draws (e.g. hemoglobin <100)
- Clinically significant kidney, heart, and hepatic impairment as determined by clinical judgement
- Taking opioid analgesics or undergoing treatment for opioid addiction
- Opioid dependence or withdrawal syndrome
- Type 1 or 2 Diabetes, or history of hypoglycemia
- Active cancer
- Baseline E+ (Na, K, Mg, phosphate) within normal range
- Baseline ALT < 60
- Baseline Hb > 100
- Baseline INR within normal range
- Baseline DFG < 50

3.5 Primary and secondary outcome measurement

3.5.1 Diagnosis of LC

There are no diagnostic tests for LC. It is a clinical condition, and the CHUS post-COVID clinic uses the WHO definition for LC (): "*Long COVID occurs in individuals with a history of probable or confirmed SARS CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms and that last for at least 2 months and cannot be explained by an alternative diagnosis. Common symptoms include fatigue, shortness of breath, cognitive dysfunction but also others and generally have an impact on everyday functioning.*

Symptoms may be new onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. All LC diagnosis at the clinic are made by an experienced clinician after ruling out other conditions.

3.5.2 Patient reported outcome measurements (PROMs)

There is no consensus definition for LC severity. For the purpose of this study, LC severity is based on the Post-COVID functional status scale (PCFS) (scale 0-4; 4 being the worse) > 2. The PCFS is part of the initial evaluation at the clinic and administered during follow up visits. There is no validated measurement instrument to assess neurocognitive impairment, but a recent Delphi consensus recommended that the Cognitive failures questionnaire (CFQ) could be used to assess cognitive impairment in patients with LC (Gorst). Performance to this test may be influence by fatigue (for example if participants have post-exertional malaise when the filled the surveys) or by depression. The CFQ is part of the initial evaluation at the clinic and administered during follow up visits.

The following patient-reported outcome measures will be used to determine pre- and post-PF health status, i.e. baseline, day 7 and 30 days:

- Post-COVID-19 functional status scale (0-4) scores: The Post-COVID Functional Status Scale (PCFS) is designed to assess the functional status and limitations of individuals experiencing LC. This PROM instrument captures the impact of LC on various aspects of daily functioning and overall well-being.[18] This instrument has been validated for patients with LC.[19]
- Fatigue: The Fatigue severity scale (FSS) (0-7 score) is one of the tool to assess the severity of fatigue. It is validated for LC patients.[20]
- SF 36: Validated scale for assessment and monitoring of patients with LC.[21] The 36-item scale's subscales (scores) are physical symptom severity (0-100), functional disability (0-50), additional symptoms (0-60), and overall health (0-10).
- Cognitive Failures Questionaire 2.0: to assess cognitive impairment (15 questions, 0-100 score). High score indicates increased propensity to cognitive impairment. The MoCa and Folstein questionnaires are not sensitive enough to capture the cognitive impairments associated with LC.[22]

3.5.3 Feasibility and acceptability

The primary objective of a pilot study, is to determine the feasibility and acceptability. To assess acceptability, participants and research staff will be asked to complete at day 30 a short questionnaire (annexe 1 and 2) about their study appreciation. The rentation rate (feasibility) will be determined at day 7 (percentage of participants that completed PF) and day 30 (percentage of participants that completed PROMs).

3.6. Study schedule

3.6.1 Study intervention

After informed consent signature, an initial nutritional evaluation will be done between 4 to 7 days prior to PF start. At baseline, participants will undergo a superved in home PF

for 7 days. Adequat hydratation, prescribed drugs already taken by the patient, and vitamins and supplements will be permitted during PF (see attach list of vitamins and supplements recommended).The vitamins (Centrum forte) will be provided to each participants. Supplements such as NaPO4, Kdur and magnesium gluconate will be prescribed to participants (all covered by RAMQ). Participants will be meet in person, at baseline, by the research nurse for detailed instructions on how to do PF (see annexe 5). The PF will done at home under medical supervision. However, the baseline visit will be done at the CRCHUS were the participant will undergo a complete medical exam by the investigator and will complete PROMs. PF will start the next day. Vitamins and supplements will be taken during PF and stop at day 7, except for thiamine which will be given until day 10.

3.6.2 Study medical supervision

The nutrionist will determine the nutritional status of participants before the baseline visit. A trained nurse will meet the participant on day 0 (baseline) and day 9. During in person visit, the nurse will take vital signs and assess the participant's well being. During PF study period, both the principal investigator and the study nutritionist will be available. At day 7, the nutritionist will contact participants to instruct them how to start refeeding.

Visit schedule:

1) The screening visit will occur between 4 to 7 days prior to the baseline visit to determine eligibility, obtain informed consent and assess nutritional status.
2) The baseline visit wiil be in person at CRCHUS, to blood draw/Sociodemographic data (Annexe 4) and PROMs/review of PF instructions (Annexe 5) (day 0).
3) PF starts the day after the baseline visit (day 1). Participants start recommended vitamins and supplements on day 1 (Annexe 3).
4) Phone follow up at day 4 and 7 for clinical assessment. End of PF at day 7. PROMs at day 7. Participants stop vitamins and supplements.
5) In person visit at CRCHUS on day 9. Blood draw during visits (E+). Refeeding syndrome surveillance
6) Phone visit visit on day 30 post-PF. PROMs and acceptability questionnaire to be completed.

At baseline, day 7 and day 30 (post-PF), participants will be asked to complete the different PROMS and sociodemographic data (baseline only) (Annexe 4). Data will be subsequently entered into a REDCap database. Patients who have a re-infection with COVID-19 during the study will follow all public health precautions and isolate as directed. In such cases, participants will be recommended to stop PF and undergo usual care for their infection.

Aside from transient minor side effects related to PF such as malaise, transient weight loss, dehydration, and fatigue, there is a small (< 1%) risk of a refeeding syndrome. Refeeding syndrome is defined as medical complications that result from fluid and

electrolyte shifts as a result of aggressive nutritional rehabilitation. ASPEN proposed unifying diagnostic criteria to stratify patients based on 3 levels of severity (mild, moderate, severe) are the following:

- Mild - a decrease in any 1, 2, or 3 of serum phosphorus, potassium, or magnesium levels by 10% to 20%
- Moderate - a decrease in any 1, 2, or 3 of serum phosphorus, potassium, or magnesium levels by 20% to 30%
- Severe - a decrease in any 1, 2, or 3 of serum phosphorus, potassium, or magnesium levels by >30% or organ dysfunction resulting from a decrease in any of these or due to thiamine deficiency (severe), occurring within 5 days of a reintroduction of calorie

This complication is mostly limited to high risk hospitalized patients. In this study, patients at high risk will be excluded (see exclusion criteria) which should considerably reduced the risk of refeeding syndrome. Although no data is available on the risk of refeeding syndrome in non-hospitalized patients, in the context of this study, the risk of refeeding syndrome is estimated to be < 0.5%. In an unlikely event of a patient presenting with a refeeding syndrome, he will be manage according to the refeeding syndrome treatment guidelines (https://myconnect.swbh.nhs.uk/wp-content/uploads/2024/02/Refeeding-guidelines.pdf). To avoid this complication, the nutritionist will instruct participants on how to start refeeding after PF.

Apart from the baseline visit which will take between 2 hours, each visit will take approximately 60 minutes or less to complete.

3.6 Study duration

We plan to complete patient accrual in about 3-6 months and expect patient participation in the last follow-up visit to be completed within 8 months. Given study interventions required, no more than two participants per week will be recruited.

4. DATA ANALYSIS

Continuous socio-demographic and clinical variables will be presented as means (standard deviation) if they follow a normal distribution, or as medians [interquartile range] otherwise. Categorical variables will be expressed as frequencies (percentages). The proportion of missing data will be reported for each variable if necessary. Variables with more than 10% missing data will be excluded from analyses. No multiple imputation is planned. A significance level of 5% will be applied, and analyses will be performed using R software (version 4.4.2).

4.1 Primary outcome

Retention rate and acceptability will be assessed using predefined calculations, with a 95% confidence interval provided to compare against unacceptable threshold. The acceptability questionnaire for participants contains two questions. Acceptability is defined as follow: « (Confiance OU Très confiance) ET (Plutôt acceptable OU Acceptable) aux deux questions ». The research staff acceptability questionnaires contains 4 questions (annexe 2). Accetability is defined as follow: « Score de 4 ou 5 sur au moins 2 des questions ».

4.2 Secondary outcomes

The within-group effect for each PROMs will be analyzed using linear mixed regression models, incorporating time as a fixed effect and patient-level variability as a random effect. Results will be reported as mean differences compared to baseline and 95% confidence intervals. Residuals normality and homoscedasticity assumptions will be validated. If violated, corrections will be made accordingly.

5. STUDY LIMITATIONS

In the absence of randomization, we cannot rule out the possibility of bias with patient selection and because of the small sample size results may not be generalizable. However, these are limitations associated with pilot studies. Sociodemographic data, including age, sex, comorbidities, initial disease severity (WHO classification), COVID vaccination status, number of infection, will be collected at baseline. No control group is included in this study.

The primary and secondary outcomes are based on PROMs which are subjective. Although the PROMs used in this study to evaluate overall health status have been validated in LC patients, their sensitivity to detect changes in the condition overtime have not been well established. Nonetheless, the use of PROMs is a common research practice for post-COVID conditions.

This pilot study in not intented to prove our hypothesis or assess the safety of the proposed intervention, but its goal is rather to enhance the rational for a larger scale study.

6. ETHICAL CONSIDERATIONS

The study will be conducted according to the current GCP, as well as the principles of the Declaration of Helsinki and its amendments. IRB committee will review and approve this protocol and informed consent. All subjects must provide a written informed consent before screening for participation in the study. This study will be performed by a qualified clinical investigator (Dr Piché) and in accordance with GCP.

The research team member will explain to the patient in lay terms the evidence that supports the study as well as the procedures, the risks and benefits, voluntary participation and the confidentiality of the study. Consenting participants will be informed that care will not be affected in any way should they decide to refuse participation or withdraw from the trial. All questions will be answered.

Given the limited funding for this study, there will be no compensation to the participants for the study visits.

6. ANTICIPATED RESULTS

If PF is found to be feasible, well-accpeted by 95% of participants and safe, , the data will form the basis for a larger prospective, multicenter, randomized clinical trial in which patients with severe LC will undergo PF to determine its clinical efficacy to improve overall health. The study will be submitted to CIHR for funding.

7. KNOWLEGE TRANSFERT

Although this is a pilot study to determine whether there is a clinical rationale to pursue further assess PF, we attempt to publish our study in a peer-reviewed journal. Although our study focus on LC patient, it could also be relevant for patients suffering from other post-infectious conditions.

8. REFERENCES

1. Al-Aly, Z., Davis, H., McCorkell, L. et al. Long COVID science, research and policy. Nat Med 30, 2148–2164 (2024). https://doi.org/10.1038/s41591-024-03173-6

2. Greenhalgh T, Sivan M, Perlowski A, Nikolich JŽ. Long COVID: a clinical update. *Lancet*. 2024;404(10453):707-724. doi:10.1016/S0140-6736(24)01136-X.

3. Statistics Canada. https://www150.statcan.gc.ca/n1/pub/75-006-x/2023001/article/00015-fra.htm

4. INSPQ.Affecion post-COVID-19 chez le personnel de la santé du Québec : fréquence,évolution et facteurs de risque. https://www.inspq.qc.ca/publications/3510

5. Mercier K, Piché J, Rioux-Perreault C, Lemaire-Paquette S, Piché A. A longitudinal prospective cohort study of health-related quality of life assessment in outpatient adults with post-COVID-19 conditions. JAMMI, 2024 Jan 16;8(4):309-31.

6. Grundler F, Mesnage R, Cerrada A, Wilhelmi de Toledo F. Improvements during long-term fasting in patients with long COVID - a case series and literature review. Front Nutr. 2023 Nov 2;10:1195270. doi: 10.3389/fnut.2023.1195270.

7. Christopher A. Rhule, Sahmla Gabriel, Toshia R. Myers, and Alan C. Goldhamer. Integrative and Complementary Therapies 2024 30:1, 11-13.

8. Davis, H.E., McCorkell, L., Vogel, J.M. *et al.* Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol* **21**, 133–146 (2023). https://doi.org/10.1038/s41579-022-00846-2

9. Al-Hakeim, H.K., Al-Rubaye, H.T., Al-Hadrawi, D.S. *et al.* Long-COVID post-viral chronic fatigue and affective symptoms are associated with oxidative damage, lowered antioxidant defenses and inflammation: a proof of concept and mechanism study. *Mol Psychiatry* **28**, 564–578 (2023). https://doi.org/10.1038/s41380-022-01836-9.

10. Tavakoli, A., Akhgarjand, C., Ansar, H. *et al.* The effects of intermittent fasting on antioxidant and inflammatory markers and liver enzymes in postmenopausal, overweight and obese women with rheumatoid arthritis: a randomized controlled trial. *Sci Rep* **15**, 2357 (2025). https://doi.org/10.1038/s41598-025-86734-0

11. Roco-Videla, Á.; Villota-Arcos, C.; Pino-Astorga, C.; Mendoza-Puga, D.; Bittner-Ortega, M.; Corbeaux-Ascui, T. Intermittent Fasting and Reduction of Inflammatory Response in a Patient with Ulcerative Colitis. *Medicina* **2023**, *59*, 1453. https://doi.org/10.3390/medicina59081453

12. Horne BD, May HT, Muhlestein JB, Le VT, Bair TL, Knowlton KU, Anderson JL Association of periodic fasting with lower severity of COVID-19 outcomes in the SARS-CoV-2 prevaccine era: an observational cohort from the INSPIRE Registry. BMJ Nutr Prev Health, 5 (2022), pp. 145-153, 10.1136/bmjnph-2022-000462

13. Mark Ezpeleta, Sofia Cienfuegos, Shuhao Lin, Vasiliki Pavlou, Kelsey Gabel, Krista A Varady, Efficacy and safety of prolonged water fasting: a narrative review of human trials, *Nutrition Reviews*, Volume 82, Issue 5, May 2024, Pages 664–675,

14. Jamshed H, Beyl RA, Della DL, Manna DL, Yang ES, Ravussin E, Peterson CM. Early time-restricted feeding improves 24-hour glucose levels and affects markers of the circadian clock, aging, and autophagy in humans
Nutrients, 11 (2019), p. 1234.

15. Antoni R., Johnston K.L., Collins A.L., Robertson M.D. Effects of intermittent fasting on glucose and lipid metabolism. Proc. Nutr. Soc. 2017;76:361–368. doi: 10.1017/S0029665116002986.

16. Wilhelmi de Toledo F., Grundler F., Sirtori C.R., Ruscica M. Unravelling the health effects of fasting: A long road from obesity treatment to healthy life span increase and improved cognition. Ann. Med. 2020;52:147–161. doi: 10.1080/07853890.2020.1770849.

17. Sugiyama, A., Takafuta, T., Sato, T. *et al.* Natural course of post-COVID symptoms in adults and children. *Sci Rep***14**, 3884 (2024). https://doi.org/10.1038/s41598-024-54397-y.

18. Klok FA, Barco S, Endres M et al. The Post-COVID-19 Functional Status scale: a tool to measure functional status over time after COVID-19. Eur Respir J. 2002 https://erj.ersjournals.com/content/56/1/2001494.

19. Ejalonibu, H., Amah, A., Aburub, A. *et al.* A review of Patient Reported Outcome Measures (PROMs) for characterizing Long COVID (LC)—merits, gaps, and recommendations. *J Patient Rep Outcomes* **8**, 101 (2024). https://doi.org/10.1186/s41687-024-00773-1.

20. Naik, H., Shao, S., Tran, K.C. *et al.* Evaluating fatigue in patients recovering from COVID-19: validation of the fatigue severity scale and single item screening questions. *Health Qual Life Outcomes* **20**, 170 (2022). https://doi.org/10.1186/s12955-022-02082-x

21. Malesevic, S., Sievi, N.A., Baumgartner, P. *et al.* Impaired health-related quality of life in long-COVID syndrome after mild to moderate COVID-19. *Sci Rep* **13**, 7717 (2023). https://doi.org/10.1038/s41598-023-34678-8

22. Bland, A.R., Barraclough, M., Trender, W.R. *et al.* Profiles of objective and subjective cognitive function in Post-COVID Syndrome, COVID-19 recovered, and COVID-19 naïve individuals. *Sci Rep* **14**, 13368 (2024). https://doi.org/10.1038/s41598-024-62050-x

23. Lewis, M., Bromley, K., Sutton, C.J. *et al.* Determining sample size for progression criteria for pragmatic pilot RCTs: the hypothesis test strikes back!. *Pilot Feasibility Stud* **7**, 40 (2021). https://doi.org/10.1186/s40814 -021-00770-x